FastPure Viral DNA/RNA Mini Kit Pro

RC323

Vazyme

Instruction for Use

Version 22.1

Vazyme

Contents

01/Product Description

This kit is suitable for the rapid extraction of high-purity viral DNA/RNA from various samples such as blood, serum, plasma, swab, tissue, bronchoalveolar lavage fluid, and cell culture supernatant. Due to the unique lysis system, the kit does not require high temperature incubation, and the addition of Carrier RNA can significantly improve the extraction efficiency of trace nucleic acids. The viral DNA/RNA can be purified quickly and efficiently by specific adsorption of silica membrane. The kit has a wide range of sample compatibility, and the obtained nucleic acid has a high purity and high yield. That nucleic acid can be directly used in downstream related experiments such as reverse transcription, PCR, fluorescence quantitative PCR, next-generation sequencing and Northern hybridization, etc.

02/Components

	Components	RC323-01 (50 rxns)
BOX 1	■ Carrier RNA (1 μg/μl)	0.6 ml
	□ Proteinase K	1.2 ml
BOX 2	Buffer VL Pro	18 ml
	Buffer VW1	27 ml
	Buffer VW2	9 ml
	RNase-free ddH_2O	6 ml
	FastPure DNA/RNA Columns (each in a 2 ml Collection Tube)	50
	RNase-free Collection Tubes 1.5 ml	50

Carrier RNA: Improve the recovery efficiency of trace nucleic acids.
Proteinase K: Digest proteins.
Buffer VL Pro: Lyse samples and provide nucleic acid binding conditions.
Buffer VW1: Remove residual proteins and other impurities.
Buffer VW2: Remove residual salt ions.
RNase-free ddH_2O: Elute DNA/RNA from adsorption column membrane.
FastPure DNA/RNA Columns: Specifically adsorb DNA/RNA.
Collection Tubes 2 ml: Collect filtrate.
RNase-free Collection Tubes 1.5 ml: Collect DNA/RNA.

03/Storage

BOX 1: Store at -30 ~ -15℃ and transported at ≤0℃.
BOX 2: Store at 15 ~ 25℃ and transported at room temperature.

04/Applications

The kit is suitable for viral DNA/RNA extraction from the following samples.

Sample Category	Sample name
Blood	whole blood, serum, plasma
Tissue	liver, spleen, lung, kidney, lymph, node, small intestine, etc
Others	stool, buccal swab, bronchoalveolar lavage fluid, cell culture supernatant

05/Self-prepared Materials

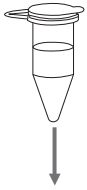
Absolute ethanol, RNase-free pipette tips, 1.5 ml RNase-free centrifuge tubes, microcentrifuge tubes, vortex mixer, manual pipettors, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

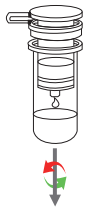
1. Before the first use, please add the specified amount of absolute ethanol to Buffer VW1 and Buffer VW2 according to the label on the bottle, and mark it.
2. Proteinase K in the kit is a cryopreserved component, and should be stored at -30 ~ -15℃ after receipt.
3. The Carrier RNA (1 μg/μl) in the kit is a cryopreserved component. Store it at -30 ~ -15℃. Please mix it well before use, distribute it into RNase-free centrifuge tubes according to the actual use situation. And avoid repeated freezing and thawing.
4. The samples should be equilibrated to room temperature in advance.
5. All procedures, unless otherwise specified, shall be carried out at room temperature.
6. The virus has a strong ability to infect, and various defense measures must be taken before operation.
7. Avoid repeated freezing and thawing of samples, otherwise the extracted viral DNA/RNA will be degraded or yield reduced.
8. When using this kit, please wear laboratory clothes, disposable latex gloves, disposable masks, and use RNase-free consumables to avoid RNase contamination to the greatest extent.

07/Mechanism & Workflow



Sample lysis: Add 20 μl Proteinase K, 300 μl sample, 300 μl Buffer VL Pro working solution (working solution preparation method: refer to 08-1/Buffer VL Pro working solution preparation) to the centrifuge tube in sequence, mix by vortexing, and incubate at room temperature for 5 min.

Adjust binding conditions: Add 200 μl absolute ethanol, vortex to mix for 15 - 30 sec, and centrifuge briefly to collect the liquid on the lid and wall of the tube.



Nucleic acid binding: Transfer the mixture to FastPure DNA/RNA Columns, and centrifuge at 12,000 rpm (13,800 × g) for 1 min.

Impurity removal: Add 700 μl Buffer VW1 (absolute ethanol has been added) to FastPure DNA/RNA Columns, and centrifuge at 12,000 rpm (13,800 × g) for 30 sec.

Add 700 μl Buffer VW2 (absolute ethanol has been added) to FastPure DNA/RNA Columns, and centrifuge at 12,000 rpm (13,800 × g) for 30 sec.

Centrifuge the empty columns at 12,000 rpm (13,800 × g) for 2 min.

Elute DNA/RNA: Add 30 - 50 μl RNase-free ddH_2O to FastPure DNA/RNA Columns, and centrifuge at 12,000 rpm (13,800 × g) for 1 min.

08/Experiment Process

08-1/Buffer VL Pro working solution preparation

Buffer VL Pro working solution is a mixture of Buffer VL Pro and Carrier RNA (1 μg/μl), please calculate according to the following formula:

X ml = n × 0.32 ml

Y μl = n × 10 μl

n: number of samples, X: required volume of Buffer VL Pro, Y: required volume of Carrier RNA

n	X	Y	n	X	Y
1	0.32 ml	10 μl	11	3.52 ml	110 μl
2	0.64 ml	20 μl	12	3.84 ml	120 μl
3	0.96 ml	30 μl	13	4.16 ml	130 μl
4	1.28 ml	40 μl	14	4.48 ml	140 μl
5	1.60 ml	50 μl	15	4.80 ml	150 μl
6	1.92 ml	60 μl	16	5.12 ml	160 μl
7	2.24 ml	70 μl	17	5.44 ml	170 μl
8	2.56 ml	80 μl	18	5.76 ml	180 μl
9	2.88 ml	90 μl	19	6.08 ml	190 μl
10	3.20 ml	100 μl	20	6.40 ml	200 μl

▲ After mixing Buffer VL Pro and Carrier RNA (1 μg/μl), gently invert up and down 10 times. To avoid foaming, do not vortex. After the working solution is prepared, it can be stored at 2 ~ 8℃ for 24 hours. It is recommended to use it now.

08-2/Sample processing

- ◆ Liquid samples such as blood, buccal swab, bronchoalveolar lavage fluid and cell culture supernatant can be directly used.
- ◆ Tissues: Put 100 mg sample into a 2 ml centrifuge tube, add 1 ml PBS or physiological saline, and homogenize until there is no obvious tissue block. Collect supernatant after brief centrifugation.
- ◆ Liquid samples such as stool swabs: Take the supernatant after brief centrifugation.

08-3/DNA/RNA extraction

The following procedures need to be carried out in the biosafety cabinet.

1. Add 20 μl Proteinase K, 300 μl sample (If the volume of sample is insufficient, use PBS or physiological saline to make up), 300 μl Buffer VL Pro working solution to the RNase-free centrifuge tube in sequence, vortex for 15 - 30 sec, and centrifuge briefly to collect the liquid on the lid and wall. Then incubate at room temperature for 5 min.
2. Add 200 μl absolute ethanol, vortex to mix for 15 - 30 sec, and centrifuge briefly to collect the liquid on the lid and wall of the tube.
   ▲Precipitation of impurities in this step is a normal phenomenon, and subsequent experiments can be carried out directly.
3. Transfer the above mixture to FastPure DNA/RNA Columns (FastPure DNA/RNA Columns have been placed in the collection tube), centrifuge at 12,000 rpm (13,800 × g) for 1 min and discard the filtrate.
4. Add 700 μl Buffer VW1 (please check first whether absolute ethanol has been added) to FastPure DNA/RNA Columns, centrifuge at 12,000 rpm (13,800 × g) for 30 sec, and discard the filtrate.
5. Add 700 μl Buffer VW2 (please check first whether absolute ethanol has been added) to FastPure DNA/RNA Columns, centrifuge at 12,000 rpm (13,800 × g) for 30 sec, and discard the filtrate.
6. Centrifuge the empty column at 12,000 rpm (13,800 × g) for 2 min.
7. Carefully transfer the FastPure DNA/RNA Columns to RNase-free Collection Tubes 1.5 ml (Provide with the kit). Add 30 - 50 μl RNase-free ddH_2O to the center of the adsorption column membrane and incubate at room temperature for 1 min. Centrifuge at 12,000 rpm (13,800 × g) for 1 min.
8. Discard the FastPure DNA/RNA Columns. The extracted DNA/RNA can be directly used for subsequent detection. Please store it at -30 ~ -15℃ for short-term storage and -85 ~ -65℃ for long-term storage.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Clogged FastPure DNA/RNA Columns	1.There are too many impurities in the sample	After centrifugation of the samples, take the supernatant for extraction.
	2.Insufficient sample lysis	After adding Buffer VL Pro working solution, shake and mix well and prolong the incubation time at room temperature to 10 min.
	3.The activity of Proteinase K is decreased	Do not premix Proteinase K with Buffer VL Pro or Buffer VL Pro working solution.
Little or no nucleic acid in eluate	1.Samples were repeatedly frozen and thawed	Use fresh samples and avoid repeated freezing and thawing.
	2.Improper storage of Carrier RNA	Please mix it well before use, distribute it into RNase-free centrifuge tubes according to the actual use situation. Store it at -30 ~ -15℃ and avoid repeated freezing and thawing.
	3.The elution process is insufficient	Add RNase-free ddH_2O to the center of the membrane and reduce the elution volume appropriately. Preheat the RNase-free ddH_2O at 65℃, extent the incubation time or perform secondary elution.
	4.The sample had not been equilibrated to room temperature	The sample shall be equilibrated to room temperature first, before being mixed with the lysis buffer and loaded on column.
	5.Improper centrifugation temperature	Please centrifuge at room temperature.
	6.Too much sediment impurities in samples such as environmental swabs affect nucleic acid binding.	Centrifuge the sample and take the supernatant to extract.
The nucleic acid does not perform well in downstream reactions	1.Residual salt ions	Add Buffer VW2 along the inner wall of the adsorption column, or after adding Buffer VW2, close the lid and invert to mix for 2 - 3 times, which will help to completely wash off the salt attached to the tube wall.
	2.Ethanol residues	After centrifuging the empty colum, place it at room temperature for 5 min to remove residual ethanol to the greatest extent.
	3.Incorrect reagents preparation	Before the first use, please add the specified amount of absolute ethanol to Buffer VW1 and Buffer VW2 according to the label on the bottle, and mark it.

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